IN THE SPECIFICATION:

Please amend the paragraph beginning on page 19, line 21 as follows:

The nucleotide sequence of this 3 cDNA clone was determined by the dideoxynucleotide chain termination method (ABI PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit, P/N 402078, Perkin Elmer) and is shown in Figure 2. The DNA fragment has a length of 460 nucleotides. The missing 5' part of the cDNA was isolated using the Marathon TM cDNA Amplification Kit of Clontech (catalog K1802-1) and following the procedure as described in the manual. Briefly, Poly A+ RNA was isolated from nectaries of *Petunia hybrida* flowers. After double stranded cDNA synthesis, adapters were ligated and a 5' RACE reaction was carried out using the adapter primer AP1 supplied in the kit and a gene-specific primer prat 122. The nucleotide sequence of prat 122 is: 5' -gtgggaaggctatgctacaagc-3' (SEQ ID NO:8) (Figure 2). The PCR product was diluted 10x and 1 µl was used in a second 5' RACE reaction with the nested adapter primer supplied by the kit (AP2) and the nested gene-specific primer prat 119 (Figure 2). The nucleotide sequence of prat 119 is: 5' -ccttctccatggactgcaatgcg-3' (SEQ ID NO:9). After gel electrophoreses a fragment of ±850 bp was obtained that hybridised with clone DD18a. The fragment, now called RC8, was extracted from the gel, purified and cloned into a PMOSBlue T-vector as described above. The sequence is shown in Figure 3. The combined (overlapping) sequences of clones DD18a and RC8 are shown in Figure 4, comprising the full length cDNA of a gene called *NEC1* hereafter. The *NEC1* clone has a length of 1205 nucleotides and encodes for a polypeptide of 265 amino acid residues. Based on the deduced amino acid

sequence, high homology was found with a cDNA that is associated with *Rhizobium*-induced nodule development in the legume *Medicago trunculata* (MtN3, gene bank number: gn1/PID/e274341). The percentages of identity and similarity are 47% and 72% respectively. Analysis of the predicted protein, using the CAOS/CAMM programme (Protein analysis 1991, Genetics Computer Group inc., Wisconsin USA), shows that the putative protein structure resembles membrane proteins, having six evenly spaced hydrophobic loops that traverse the cell membrane. In addition, a signal sequence is predicted at the N-terminus, while the C-terminus is highly hydrophilic. Highest homology with MtN3 is found in the N-terminal signal sequence, the first two membrane-spanning loops and the last two membrane-spanning loops. The C-terminal hydrophilic part shows the lowest homology (28% identity, 30% similarity). The function of *NEC1* has not yet been determined.

Please amend the paragraph beginning on page 22, line 17 as follows:

The RNA expression of *NEC1* was determined by standard Northern blot hybridisation experiments. A DNA fragment comprising the complete sequence of the Differential Display clone DD18 (Figure 2) was used as a probe. Using 10 μg of total RNA from various petunia tissues, strong expression of *NEC1* was detectable in nectaries and weak expression in anthers. No expression was detectable in other floral organs, in leaves or in roots (Figure 5A).

The expression in the ovary and nectaries was determined by in situ hybridisation using a DIG labelled antisense RNA probe corresponding to the nucleotides 79 to 1036 of *NEC1* cDNA, comprising the coding region and part of the 3 untranslated region. A clone containing this sequence was obtained by PCR on adapter-ligated cDNA, using two gene-specific primers prat 122 and prat 129 (Figure 4). The nucleotide sequence of prat 122 is: 5' -gtgggaaggctatgctacaagc-

3' (SEQ ID NO:8), comprising the nucleotides 1015 to 1036 of the *NEC1* cDNA. The nucleotide sequence of prat 129 is: 5' - gggatccatggcgcaattacgtgctgatg-3' (SEQ ID NO:10), comprising the nucleotides 79 to 100 of the *NEC1* cDNA. The gene-specific region of the primers is underlined. The primer contains an extra BamHI and NcoI site at the 5' end. A PCR fragment of 958 nucleotides was obtained and cloned into a PMOSBlue vector. The fragment was subcloned in a vector containing the T7 promoter and in vitro antisense RNA transcripts were made using T7 RNA polymerase. A standard protocol for in situ hybridisation was used as described by Canãs et al., 1994. Strong hybridizing signals were observed in the outer cell layers of the nectaries (Figure 6A)

Please amend the paragraph beginning on page 23, line 16 as follows:

The promoter fragment of *NEC1* was cloned using the genome walker protocol (PT3042-1) and kit as provided by Clontech Laboraties. Briefly, genomic DNA from *Petunia hybrida* was digested with 5 different blunt cutting restriction enzymes. Genome Walker adapters were ligated and PCR reactions were carried out on each Genome Walker "library" with a gene specific, reversed primer prat 148 and the adapter primer from the kit (AP1). The nucleotide sequence of prat 148 is: 5' - ccaagaaggccaaatatgaaagac-3' (SEQ ID NO:11) comprising the nucleotides 105 to 128 of the *NEC1* cDNA (Figure 4). PCR products were subjected to a second round of PCR, using the nested adapter primer AP2 and the nested gene specific, reversed primer prat 149. The nucleotide sequence of prat 149 is: 5' -aagtcatcagcacgtaattgcgcc-3' (SEQ ID NO:12), comprising the nucleotides 81 to 104 of the *NEC1* cDNA. From the second PCR a 2 kb fragment was isolated from the *Stu*I library, which was cloned in the PMOSBlue T-vector, yielding the

construct pMA5-10. Figure 7 (SEQ ID NO:7) shows the DNA sequence of the *NEC1* promoter in the construct pMA5-10, including the translation start of *NEC1* cDNA.

Please amend the paragraph beginning on page 24, line 4 as follows:

A PCR reaction was performed on pMA5-10 (Example 5), using the forward vector primer U19 of pMOSBlue and the gene-specific primer prat 169. The nucleotide sequence of prat 169 is:

5' -cgctgcagcgccatggttttttttagtgaagcccc-3' (SEQ ID NO:13). The gene-specific region is underlined. The primer contains an NcoI and BglII restriction site at the 3 end. The PCR product was digested with KpnI and NcoI and ligated into a pBluescript-derived vector (pMO4) that contains the NTM19 promoter (Custers et al., 1997), the reporter gene *GUS* and the nos terminator. The KpnI/NcoI NTM19 promoter fragment was replaced, resulting in a *NEC1*-promoter/*GUS* translational fusion. The resulting plasmid pNEP1 was digested with SmaI to release the *NEC1* promoter/*GUS*/nos fragment and this fragment was ligated into a derivative of the binary plasmid pBIN (Bevan, 1984) yielding the binary plasmid pBNEP1 (Figure 8). pBNEP1 was introduced into *Agrobacterium tumefaciens* strain LBA4404 or C58pMP90 by electroporation. Plasmid DNA from the Agrobacterium transformants was isolated and the structure of the binary vector was verified by restriction analysis and PCR.

Please amend the paragraph beginning on page 26, line 22 as follows:

Honey samples were loaded on an SDS PAGE gel and after electrophoreses the gel was blotted on a PVDF membrane. After staining the CVH29 and CVH50 bands were cut out from

the blot and N-terminal sequencing was performed on both proteins. The N-terminal sequence of CVH50 is: SVLDFCVADPSLPDGPAGYSCTEPSTVTSQDF (SEQ ID NO:14). The N-terminal sequence of CVH29 is: SVLDFCVADPS-LPDGPAGYSCKEPAKVTVDDFVFHGLGTA (SEQ ID NO:15). A gene bank homology search (BLAST) showed high amino acid sequence homology (63%) with germin-like proteins isolated from Arabidopsis (Figure 12).

Please amend the paragraph beginning on page 27, line 4 as follows:

Because the germin-like protein CVH29 is excreted in heather nectar it was expected that part of the cDNA encodes a signal sequence. Based on the N-terminal amino acid sequence, degenerated primers were designed. The sequence of the forward primer prat 176 is: 5' - gayttytgygtngcngaycc-3' (SEQ ID NO:16) (y= c or t, n= c, t, a or g). The sequence of the reversed primer prat 177 is: ccrtgraanacraartcrtc (SEQ ID NO:17) (r= g or a). A PCR reaction performed on genomic DNA of heather yielded a 99 bp DNA fragment. The fragment was sequenced and two reversed, gene-specific 5 primers were designed to clone the 5 cDNA by Marathon cDNA racing using the kit and protocol of Clontech laboratories (protocol PT1115-1, Clontech Palo Alto USA). The sequence of gene-specific primer prat 207 that was used is: 5' - ggtgactttagagggctccttgc-3' (SEQ ID NO:18), the sequence of gene-specific nested primer prat 206 is:

5' -gctccttgcaggagtagcctgc-3' (SEQ ID NO:19) (Figure 13). RNA was isolated from open flowers of heather and mRNA was prepared using the Pharmacia quickprep micro mRNA kit. After cDNA synthesis and adapter ligation a PCR reaction was performed, using the adapter primer

AP1 and the gene-specific primer prat 207. The PCR product was used for a second PCR, using adapter primer AP2 and the nested gene-specific primer prat 206. A single fragment of around 300 nucleotides was obtained and cloned in a PMOSBlue T-vector. Four clones were sequenced. Figure 14 shows that three clones were identical and one clone had two different nucleotides in the untranslated 5 region. A putative signal sequence of 17 amino acids was identified between the ATG start codon and the first codon of the mature protein CVH29 that was identical in all four clones. The nucleotide sequence of the putative signal sequence (SEQ ID NO:6) is:

5' -atgtttcttccaattctcttcaccatttccctcctcttctcctcctcccatgct-3' .

Please amend the paragraph beginning on page 28, line 5 as follows:

To clone the *NEC1* promoter into a PMOSBlue vector a PCR reaction was carried out on pMA5-10 (example 5) using the forward primer prat 247 and the reversed primer prat 248 (Fig. 7). Prat 247 contains an extra Pst1 restriction site. The *Nde*I restriction site of prat 248 coincides with the ATG translation start of *NEC1*. The nucleotide sequence of prat 247 is: 5' - ggctgcaggagtgttctttgatagaatg-3' (SEQ ID NO:20), the nucleotide sequence of prat 248 is: 5' - cgccatatgttttttatggaagcccc-3' (SEQ ID NO:21). Gene-specific regions are underlined. A 1,8 kb promoter fragment was obtained and cloned into a pMOSBlue vector, yielding the plasmid pNECP.

Please amend the paragraph beginning on page 28, line 17 as follows:

A DNA molecule encoding the signal sequence CVSP as depicted in SEQ ID NO:6 was produced by synthesis and subsequent annealing of two oligo molecules prat 245 and prat 246. The sequence of prat 245 is: 5' tatgttccttccaattcttttcactatttct-cttcttttctcttcttctcatgcttctgttcttgatttc' 3 (SEQ ID NO:22), the sequence of prat 246 is: 5' gatccgaaatcaagaacagaagcatgagaagaagagaaaagaa-gagaaatagtgaaaagaattggaaggaaca'3 (SEQ ID NO:23). The region encoding the signal sequence CVSP is underlined. To ensure correct cleavage of the signal peptide, the linkers were extended with the coding region for the first five amino acids of the mature germin-like protein (Fig. 13). The codon usage of the signal peptide sequence was optimised for Arabidopsis. By addition of a BamHI restriction site at the 3 end, 2 extra amino acids were linked in frame to the mature protein. The resulting DNA molecule is shown in Figure 15. The fragment was ligated into a Nde1/BamH1 cut PMOSBlue vector, yielding the plasmid pCVSP.

Please amend the paragraph beginning on page 29, line 4 as follows:

A 250 bp long fragment containing the NOS terminator sequence (NOST) was obtained by PCR, using the forward primer prat 251 and the reversed primer prat 252 on DNA of pRAP 33, which is a pUC 19 derived plasmid. Prat 251 adds a SacI and XhoI site, prat 252 adds a SmaI and EcoRI site. The sequence of prat 251 is: 5' -gggagctcgagtcgttcaaacatttggcaataaag-3' (SEQ ID NO:24). The sequence of prat 252 is: 5' -cgaattcccgggatctagtaacatagatgacac-3' (SEQ ID NO:25). The NOST-specific regions are underlined. The PCR product was cloned into pCR-Script™ Amp SK(+) Cloning Kit (Catalog 21188-21190, Stratagene Al Jolla USA), yielding the plasmid pCR-

NOST. pCR-NOST was digested with Sac1 and EcoR1 and the resulting fragment was cloned into the pUC 19 (ClonTech), derived plasmid pUCAP yielding the plasmid pCVNOS.

Please amend the paragraph beginning on page 29, line 19 as follows:

The plasmid pGUSN358 was purchased from Clontech (catalog 6030-1) containing the reporter gene GUS in pUC 119, modified to destroy the N-linked glycosylation site within the 1.814 Kb GUS coding sequence. A PCR reaction was carried out with gene-specific primers prat 249 and prat 250, yielding a fragment that contains the GUS gene coding region and a BamH1 restriction site at the 5 end and a Sac1 restriction site at the 3 end. The sequence of prat 249 is: 5' -ccggatccatgttacgtcctgtagaaacc-3' (SEQ ID NO:26). The sequence of prat 250 is: 5' -gggagctcccaccgaggctgtag-3' (SEQ ID NO:27). The GUS specific regions are underlined. Subsequently, the PCR fragment was digested with BamHI and SacI and ligated into the BamHI/SacI cut plasmid pCVNOS, yielding the plasmid pCV2. A schematic representation of pCV2 is given in Figure 17.

Please amend the paragraph beginning on page 34, line 5 as follows:

PCR primers were designed that hybridise with the cDNA of an invertase gene cloned from *Solanum tuberosum*. The 5' primer 5' -AAGGACTTTAGAGAGACCCGACCACTGCTGG-3' (SEQ ID NO:28) and the 3' primer 5' -AAATGTCTTTGATGCATAATATTTCCCATAATC-3' (SEQ ID NO:29) were used for a PCR reaction on genomic DNA of petunia to yield a fragment of around 420 bp. The fragment was

sequenced and cloned into a pMOSBlue vector to used as a probe to screen a petunia nectary-specific cDNA library. Hybridizing phage plaques were purified and cDNAs were retrieved by in vivo excision as described in example 2. The expression of the cDNA s was determined by Northern blotting as described in example 3 and the sequence of a nectary-specific invertase was determined as described in example 2. The invertase gene was amplified using a 5' primer that hybridises with sequences just upstream of the ATG translation start site and a 3' primer that hybridises with sequences just downstream of the translation stop site. Extra restriction enzyme recognition sites were generated to allow cloning of the cDNA in sense (overexpression) or antisense direction into the binary vector pCPO31 as described in example 18. The chimerical gene constructs are transferred via Agrobacterium GV3101 to petunia variety W115, using the transformation method as described in example 7. Transgenic petunia plants were selected that exhibit modified sugar composition in nectar.

The nucleotide sequence of this 3 cDNA clone was determined by the dideoxynucleotide chain termination method (ABI PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit, P/N 402078, Perkin Elmer) and is shown in Figure 2. The DNA fragment has a length of 460 nucleotides. The missing 5' part of the cDNA was isolated using the Marathon TM cDNA Amplification Kit of Clontech (catalog K1802-1) and following the procedure as described in the manual. Briefly, Poly A+ RNA was isolated from nectaries of *Petunia hybrida* flowers. After double stranded cDNA synthesis, adapters were ligated and a 5' RACE reaction was carried out using the adapter primer AP1 supplied in the kit and a gene-specific primer prat 122. The nucleotide sequence of prat 122 is: 5' -gtgggaaggctatgctacaagc-3' (SEQ ID NO:8) (Figure 2). The PCR product was diluted 10x and 1 µl was used in a second 5' RACE reaction with the nested adapter primer supplied by the kit (AP2) and the nested gene-specific primer prat 119 (Figure 2). The nucleotide sequence of prat 119 is: 5'-ccttctccatggactgcaatgcg-3 (SEQ ID NO:9). After gel electrophoreses a fragment of ±850 bp was obtained that hybridised with clone DD18a. The fragment, now called RC8, was extracted from the gel, purified and cloned into a PMOSBlue T-vector as described above. The sequence is shown in Figure 3. The combined (overlapping) sequences of clones DD18a and RC8 are shown in Figure 4, comprising the full length cDNA of a gene called *NEC1* hereafter. The *NEC1* clone has a length of 1205 nucleotides and encodes for a polypeptide of 265 amino acid residues. Based on the deduced amino acid sequence, high homology was found with a cDNA that is associated with *Rhizobium*-induced nodule development in the legume *Medicago trunculata* (MtN3, gene bank number: gn1/PID/e274341). The percentages of identity and similarity are 47% and 72% respectively. Analysis of the predicted protein, using the CAOS/CAMM programme (Protein analysis 1991, Genetics Computer Group inc., Wisconsin USA), shows that the putative protein structure resembles membrane proteins, having six evenly spaced hydrophobic loops that traverse the cell

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169 is:

5' -cgctgcagcgccatggttttttttagtgaagcccc-3' (SEQ ID NO:13). The gene-specific region is underlined. The primer contains an NcoI and BglII restriction site at the 3 end. The PCR product was digested with KpnI and NcoI and ligated into a pBluescript-derived vector (pMO4) that contains the NTM19 promoter (Custers et al., 1997), the reporter gene *GUS* and the nos terminator. The KpnI/NcoI NTM19 promoter fragment was replaced, resulting in a *NEC1*-promoter/*GUS* translational fusion. The resulting plasmid pNEP1 was digested with SmaI to release the *NEC1* promoter/*GUS*/nos fragment and this fragment was ligated into a derivative of the binary plasmid pBIN (Bevan, 1984) yielding the binary plasmid pBNEP1 (Figure 8). pBNEP1 was introduced into *Agrobacterium tumefaciens* strain LBA4404 or C58pMP90 by electroporation. Plasmid DNA from the Agrobacterium transformants was isolated and the structure of the binary vector was verified by restriction analysis and PCR.

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Honey samples were loaded on an SDS PAGE gel and after electrophoreses the gel was blotted on a PVDF membrane. After staining the CVH29 and CVH50 bands were cut out from the blot and N-terminal sequencing was performed on both proteins. The N-terminal sequence of CVH50 is: SVLDFCVADPSLPDGPAGYSCTEPSTVTSQDF (SEQ ID NO:14). The N-terminal sequence of CVH29 is: SVLDFCVADPS-LPDGPAGYSCKEPAKVTVDDFVFHGLGTA (SEQ ID NO:15). A gene bank homology search (BLAST) showed high amino acid sequence homology (63%) with germin-like proteins isolated from Arabidopsis (Figure 12).

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5' -gctccttgcaggagtagcctgc-3' (SEQ ID NO:19) (Figure 13). RNA was isolated from open flowers of heather and mRNA was prepared using the Pharmacia quickprep micro mRNA kit. After cDNA synthesis and adapter ligation a PCR reaction was performed, using the adapter primer AP1 and the gene-specific primer prat 207. The PCR product was used for a second PCR, using adapter primer AP2 and the nested gene-specific primer prat 206. A single fragment of around 300 nucleotides was obtained and cloned in a PMOSBlue T-vector. Four clones were sequenced. Figure 14 shows that three clones were identical and one clone had two different nucleotides in the untranslated 5 region. A putative signal sequence of 17 amino acids was identified between the ATG start codon and the first codon of the mature protein CVH29 that was identical in all four clones. The nucleotide sequence of the putative signal sequence (SEQ ID NO:6) is:

5' -atgtttcttccaattctcttcaccatttccctcctcttctcctcctcccatgct-3' .

Please amend the paragraph beginning on page 28, line 5 as follows:

To clone the *NEC1* promoter into a PMOSBlue vector a PCR reaction was carried out on pMA5-10 (example 5) using the forward primer prat 247 and the reversed primer prat 248 (Fig. 7). Prat 247 contains an extra Pst1 restriction site. The *Nde*I restriction site of prat 248 coincides with the ATG translation start of *NEC1*. The nucleotide sequence of prat 247 is: 5' - ggctgcaggagtgttctttgatagaatg-3' (SEQ ID NO:20), the nucleotide sequence of prat 248 is: 5' - cgccatatgttttttttatggaagcccc-3' (SEQ ID NO:21). Gene-specific regions are underlined. A 1,8 kb promoter fragment was obtained and cloned into a pMOSBlue vector, yielding the plasmid pNECP.

Please amend the paragraph beginning on page 28, line 17 as follows:

A DNA molecule encoding the signal sequence CVSP as depicted in SEQ ID NO:6 was produced by synthesis and subsequent annealing of two oligo molecules prat 245 and prat 246. The sequence of prat 245 is: 5' tatgttccttccaattcttttcactatttct-cttcttttctcttcttctcatgcttctgttcttgatttc' 3 (SEQ ID NO:22), the sequence of prat 246 is: 5' gatccgaaatcaagaacagaagcatgagaagaagagaaaagaa-gagaaatagtgaaaagaattggaaggaaca'3 (SEQ ID NO:23). The region encoding the signal sequence CVSP is underlined. To ensure correct cleavage of the signal peptide, the linkers were extended with the coding region for the first five amino acids of the mature germin-like protein (Fig. 13). The codon usage of the signal peptide

sequence was optimised for Arabidopsis. By addition of a BamHI restriction site at the 3 end, 2 extra amino acids were linked in frame to the mature protein. The resulting DNA molecule is shown in Figure 15. The fragment was ligated into a Nde1/BamH1 cut PMOSBlue vector, yielding the plasmid pCVSP.

Please amend the paragraph beginning on page 29, line 4 as follows:

A 250 bp long fragment containing the NOS terminator sequence (NOST) was obtained by PCR, using the forward primer prat 251 and the reversed primer prat 252 on DNA of pRAP 33, which is a pUC 19 derived plasmid. Prat 251 adds a SacI and XhoI site, prat 252 adds a SmaI and EcoRI site. The sequence of prat 251 is: 5' -gggagctcgagtcgttcaaacatttggcaataaag-3' (SEQ ID NO:24). The sequence of prat 252 is: 5' -cgaattcccgggatctagtaacatagatgacac-3' (SEQ ID NO:25). The NOST-specific regions are underlined. The PCR product was cloned into pCR-Script™ Amp SK(+) Cloning Kit (Catalog 21188-21190, Stratagene Al Jolla USA), yielding the plasmid pCR-NOST. pCR-NOST was digested with Sac1 and EcoR1 and the resulting fragment was cloned into the pUC 19 (ClonTech), derived plasmid pUCAP yielding the plasmid pCVNOS.

Please amend the paragraph beginning on page 29, line 19 as follows:

The plasmid pGUSN358 was purchased from Clontech (catalog 6030-1) containing the reporter gene GUS in pUC 119, modified to destroy the N-linked glycosylation site within the 1.814 Kb GUS coding sequence. A PCR reaction was carried out with gene-specific primers prat 249 and prat 250, yielding a fragment that contains the GUS gene coding region and a BamH1

restriction site at the 5 end and a Sac1 restriction site at the 3 end. The sequence of prat 249 is: 5' -ccggatccatgttacgtcctgtagaaacc-3' (SEQ ID NO:26). The sequence of prat 250 is: 5' -gggagctccaccgaggctgtag-3' (SEQ ID NO:27). The GUS specific regions are underlined. Subsequently, the PCR fragment was digested with BamHI and SacI and ligated into the BamHI/SacI cut plasmid pCVNOS, yielding the plasmid pCV2. A schematic representation of pCV2 is given in Figure 17.

Please amend the paragraph beginning on page 34, line 5 as follows:

PCR primers were designed that hybridise with the cDNA of an invertase gene cloned from *Solanum tuberosum*. The 5 primer 5' -AAGGACTTTAGAGAGACCCGACCACTGCTGG-3' (SEQ ID NO:28) and the 3' primer 5' -AAATGTCTTTGATGCATAATATTTCCCATAATC-3' (SEQ ID NO:29) were used for a PCR reaction on genomic DNA of petunia to yield a fragment of around 420 bp. The fragment was sequenced and cloned into a pMOSBlue vector to used as a probe to screen a petunia nectary-specific cDNA library. Hybridizing phage plaques were purified and cDNAs were retrieved by in vivo excision as described in example 2. The expression of the cDNA s was determined by Northern blotting as described in example 3 and the sequence of a nectary-specific invertase was determined as described in example 2. The invertase gene was amplified using a 5' primer that hybridises with sequences just upstream of the ATG translation start site and a 3' primer that hybridises with sequences just downstream of the translation stop site. Extra restriction enzyme recognition sites were generated to allow cloning of the cDNA in sense (overexpression) or antisense direction into the binary vector pCPO31 as described in example 18. The chimerical